View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Resolution of Psoriasis by a Leukocyte-Targeting Bacterial Protein in a Humanized Mouse Model

Karin Stenderup[1], Cecilia Rosada[1], Thomas N. Dam[2], Erica Salerno[3], Benjamin A. Belinka[4] and Scott C. Kachlany[3,4]

Psoriasis is a very common chronic skin disease, affecting 2–3% of the world's population or more than 125 million individuals worldwide. The characteristic lesion of psoriasis is due to rapid proliferation and shortened transition of keratinocytes through the epidermis. Proinflammatory white blood cells (WBCs) migrate into the psoriatic plaques, and the pathogenic cytokine environment causes the changes in keratinocyte proliferation and differentiation. Enhanced migration of WBCs is due to the upregulation and activation of adhesion molecules such as leukocyte function antigen-1 (LFA-1), which binds intercellular adhesion molecule-1 (ICAM-1) on endothelial cells. Targeting LFA-1 and preventing interaction with ICAM-1 has proven an effective strategy for treating psoriasis. We show here that a natural leukocyte-targeting bacterial protein (leukotoxin (LtxA)) that binds LFA-1 can inhibit proliferation of activated WBCs from psoriasis patients and demonstrates significant therapeutic efficacy in a psoriasis xenograft transplantation model. In *ex vivo* studies, LtxA preferentially targeted proinflammatory WBC subtypes, including activated $CD25^+$ T cells and $CD14^+CD16^+$ monocytes. LFA-1 has been shown to have a significant role in the pathogenesis of numerous autoimmune and inflammatory diseases, and we propose that LtxA may be a highly effective agent for treating these diseases.

Journal of Investigative Dermatology (2011) **131,** 2033–2039; doi:10.1038/jid.2011.161; published online 9 June 2011

INTRODUCTION

Psoriasis is one of the most prevalent inflammatory skin diseases, afflicting 2–3% of the population or 125 million people worldwide (Nestle *et al.*, 2009; Valdimarsson *et al.*, 2009). It is well established that psoriasis is a lifelong and complex disease with a hereditary basis. The disease is fundamentally an inflammatory skin condition that results from a dysregulated immune response, leading to the activation of immune cells in the skin and resulting in an abnormal proliferation and differentiation of epidermal keratinocytes. At the skin surface, differentiated keratinocytes manifest as elevated and scaly lesions that vary in size from <1 cm to several centimeters. The thickened epidermis, expanded dermal vascular compartment, and infiltrate of immunocompetent cells in both the dermis and the epidermis account for the characteristic psoriatic lesions.

[1]*Department of Dermatology, Aarhus University Hospital, Aarhus, Denmark;* [2]*Department of Dermatology, Roskilde Hospital, Roskilde, Denmark;* [3]*Department of Oral Biology, New Jersey Dental School, University of Medicine and Dentistry of New Jersey, Newark, New Jersey, USA and* [4]*Actinobac Biomed, North Brunswick, New Jersey, USA*

Correspondence: Scott C. Kachlany, Department of Oral Biology, New Jersey Dental School, University of Medicine and Dentistry of New Jersey, 185 South Orange Avenue, Medical Science Building C636, Newark, New Jersey, USA. E-mail: kachlasc@umdnj.edu

Abbreviations: ICAM-1, intercellular adhesion molecule-1; LFA-1, leukocyte function antigen-1; LtxA, leukotoxin; PBMC, peripheral blood mononuclear cell; VLA-1, very late antigen-1; WBC, white blood cell

Received 13 January 2011; revised 23 March 2011; accepted 24 April 2011; published online 9 June 2011

Studies have shown that the white blood cells (WBCs) involved in psoriasis have an upregulation and activation of leukocyte function antigen-1 (LFA-1) (McGregor *et al.*, 1992; de Boer *et al.*, 1994). LFA-1 is a β2-integrin on the surface of WBCs composed of CD11a and CD18, and involved in immune cell migration and signaling (Kinashi, 2005). LFA-1 is also involved in cellular activation and stimulation through interaction with its natural ligand, intercellular adhesion molecule-1 (ICAM-1) (Abraham *et al.*, 1999; Camacho *et al.*, 2001; Chirathaworn *et al.*, 2002; Kandula and Abraham, 2004). LFA-1 can exist in multiple states, including an activated form and an inactive form (Hogg *et al.*, 1993; Hogg *et al.*, 2004). LFA-1 in the activated form binds ICAM-1 on endothelial cells and leads to extravasation of WBCs into tissue. Efalizumab, a recombinant monoclonal antibody that binds to the CD11a subunit of LFA-1, was approved for clinical use in patients with psoriasis but was later withdrawn from the market because of increased risk of viral infection of the central nervous system (progressive multifocal leukoencephalopathy; Sterry *et al.*, 2009).

Leukotoxin (LtxA) is a ~113-kDa protein produced by the Gram-negative oral bacterium *Aggregatibacter actinomycetemcomitans* (reviewed recently in Kachlany (2010)) and binds specifically to LFA-1 (Lally *et al.*, 1997). After binding LFA-1, LtxA kills WBCs of humans and old-world primates by inducing apoptosis at low doses and necrosis at high doses (Korostoff *et al.*, 1998; Korostoff *et al.*, 2000). Cells that lack LFA-1 are resistant to killing by LtxA, and we recently showed that LtxA preferentially targets cells with high amounts of activated LFA-1 (Kachlany *et al.*, 2010). Thus, LtxA kills only a subset of WBCs, without affecting other cells or organs in

© 2011 The Society for Investigative Dermatology

the body. Because of the high degree of specificity of the native protein, we have been investigating the potential therapeutic utility of LtxA for targeting WBC diseases. In preclinical animal studies, LtxA was shown to be highly effective in treating leukemia, and when injected in a non-human primate, LtxA was active, specific, and well tolerated (Kachlany *et al.*, 2010). After administration of LtxA to the non-human primate, we observed partial (~50%) depletion of only WBCs and the drop was transient (Kachlany *et al.*, 2010), suggesting that prolonged immunosuppression would not occur.

There is no cure for psoriasis, but current treatments for mild-to-moderate psoriasis include topical therapy and phototherapy. Systemic administration of retinoids, methotrexate, and cyclosporine has mainly been reserved for moderate and severe cases because of associated risks and known adverse reactions (Kunz, 2009; Poulin *et al.*, 2009). Over the last decade, targeted therapy in the form of biologics has shown many advantages over the 'traditional systemics' and exhibit fewer side effects. In this report, we tested LtxA against activated WBCs from psoriasis patients and in a humanized animal model for psoriasis.

RESULTS

Effect of LtxA and efalizumab on proliferation of activated PMBCs from psoriasis patients

To determine the activity of LtxA on activated peripheral blood mononuclear cells (PBMCs) from psoriasis patients, we isolated PBMCs from 10 donors with severe plaque psoriasis (3 women and 7 men, age 29–63 years (average 48 ± 13 years)). PBMCs were isolated from blood samples by centrifugation over a lymphoprep density gradient (Sigma-Aldrich, Broenby, Denmark) and then activated using staphylococcal enterotoxin B (Sigma-Aldrich) and treated with dilutions of LtxA or efalizumab. We found that both LtxA and efalizumab inhibited proliferation of the activated PBMCs from psoriasis patients, but the effective doses of LtxA were ~16,000 times lower than those of efalizumab (Figure 1).

Establishment of the psoriasis xenograft transplantation model

We wished to determine whether LtxA has the ability to alleviate symptoms of psoriasis *in vivo*. For these studies, we used the established humanized mouse model where human psoriatic plaques are transplanted onto severe combined immunodeficient mice (Dam *et al.*, 1999; Villadsen *et al.*, 2003; Villadsen *et al.*, 2007; Stenderup *et al.*, 2009; Jakobsen *et al.*, 2009; Rosada *et al.*, 2010). Skin biopsies from two patients with severe plaque-type psoriasis were grafted onto mice and LtxA ($0.5\,mg\,kg^{-1}$ ($4.4\,nmol\,kg^{-1}$)), efalizumab ($6\,mg\,kg^{-1}$ ($40\,nmol\,kg^{-1}$)), or vehicle (tris buffer/NaCl) was administered once daily by intraperitoneal injection for 3 weeks. Over the 3-week period, mice treated with LtxA did not show any adverse physiological changes, and their bodyweight increased similarly to the vehicle-treated mice (data not shown).

Clinical assessment of psoriatic plaques

Semiquantitative clinical psoriasis scores were recorded twice weekly throughout the study. Assessment was based on a scale of 0–3, where 3 represents full psoriasis and 0 represents lack of disease. LtxA significantly decreased the semiquantitative clinical psoriasis score ($P<0.001$) after 3 weeks of treatment (Figure 2a). Efalizumab (at nine times higher concentration ($mol\,kg^{-1}$) than LtxA) also decreased the clinical psoriasis score ($P=0.094$) after 3 weeks, however, not to a significant degree (Figure 2a).

Epidermal thickness was measured on hematoxylin and eosin-stained paraffin-embedded sections after 3 weeks of treatment. LtxA (45%; $P=0.002$) and efalizumab (35%; $P=0.024$) significantly decreased the epidermal thickness (Figure 2b and c).

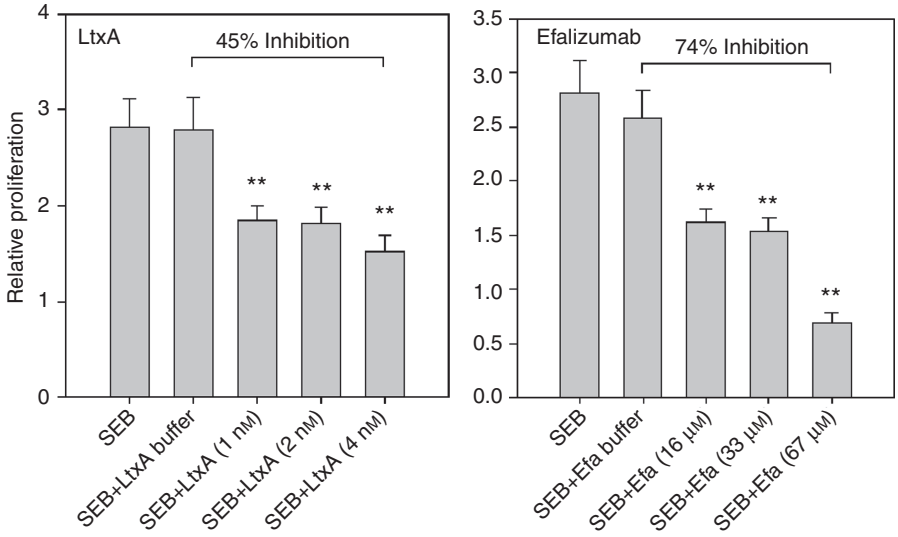

Figure 1. Effect on proliferation of white blood cells from psoriasis patients. Peripheral blood mononuclear cells were isolated from 10 psoriasis patients, activated with staphylococcal enterotoxin B (SEB), and treated with different doses of leukotoxin (LtxA) or efalizumab (Efa) for 72 hours. Proliferation was measured using an ATP-based assay comparing activated cultures with non-activated.

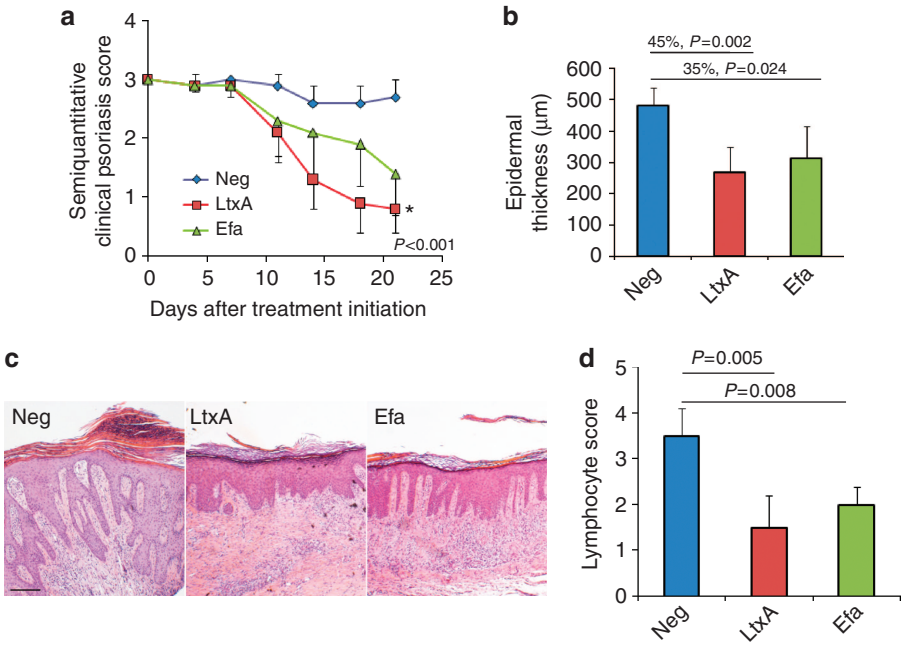

Figure 2. Effect of treatment on human xenograft transplants. (**a**) Clinical psoriasis assessment. Psoriatic plaque transplants on severe combined immunodeficient mice were evaluated twice weekly using a scale of 0–3. Treatment was vehicle (neg), leukotoxin (LtxA), or efalizumab (Efa) daily for 3 weeks. (**b**) Effect of treatment on epidermal thickness of psoriatic plaques. After 3 weeks of treatment, epidermal sections of transplants were stained with hematoxylin and eosin (H&E) and the thickness (μm) was determined. (**c**) Representative histology of H&E-stained paraffin-embedded sections. Bar = 200 μm. (**d**) Assessment of lymphocyte infiltration in transplants. H&E-stained epidermal sections were evaluated for the histological presence of lymphocytes after 3 weeks of treatment. A score of 4 represents maximum lymphocyte infiltration and 0 represents baseline infiltration. Shown is mean ± SEM. Efa, efadizumab; neg, negative.

Psoriatic skin is characterized by an increased infiltration of lymphocytes compared with healthy skin. To determine the pathological effect of LtxA therapy, we determined the lymphocyte score. The score is an assessment of the degree of lymphocytic infiltrate present both in the dermal and the epidermal compartment. LtxA ($P=0.005$) and efalizumab ($P=0.008$) both significantly decreased the degree of lymphocytic infiltration after 3 weeks of treatment (Figure 2d). Evaluation of skin biopsies was performed randomly in a blinded manner.

Sensitivity of activated T cells to LtxA

We previously showed that a Jurkat T-cell line expressing a constitutively activated form of LFA-1 was ~10 times more sensitive to LtxA than parental wild-type cells (Kachlany *et al.*, 2010). We wished to determine whether primary T cells that are naturally activated are also more susceptible to LtxA-mediated apoptosis. We treated human PBMCs with LtxA and then determined the levels of killing of activated $CD4^+CD25^+$ cells and resting $CD4^+CD25^-$ cells. We found that activated $CD25^+$ T cells from three different donors were consistently more sensitive to LtxA than resting cells ($CD4^+CD25^-$; Figure 3a). The percentage of apoptosis of $CD4^+CD25^+$ cells ranged from 21 to 190% greater than $CD4^+CD25^-$ cells.

Effect of LtxA on $CD16^+$ monocytes

In addition to lymphocytes, monocytes/macrophages that migrate from circulation into the tissue have a critical role in the pathogenesis of psoriasis (van den Oord and de Wolf-Peeters, 1994; Nickoloff, 2000; Vestergaard *et al.*, 2004). Specifically, $CD16^+$ proinflammatory monocytes are increased in patients with autoimmune disease and inflammation (Fingerle *et al.*, 1993; Baeten *et al.*, 2000; Todd *et al.*, 2007; Ziegler-Heitbrock, 2007; Chiu *et al.*, 2010). To determine whether LtxA also has an effect on proinflammatory monocytes, we tested the activity of LtxA on peripheral blood, gating for $CD14^+CD16^+$ monocytes. We found that primary $CD16^+$ monocytes were highly sensitive to LtxA in a dose-dependent manner, as determined by Annexin V staining and flow cytometry (Figure 3b).

DISCUSSION

In this paper, we show that a natural bacterial protein with specificity for WBCs is highly effective in inhibiting proliferation of activated WBCs from psoriasis patients and promoting resolution of psoriasis in a psoriasis xenograft transplantation model. The receptor for LtxA, LFA-1, has been previously validated as an effective target for treatment of psoriasis in both animal models (Zeigler *et al.*, 2001) and humans (Jullien *et al.*, 2004; Harper *et al.*, 2008).

Lymphocytes, and specifically T cells, are believed to be the critical cell type for initiating and perpetuating psoriasis (Wrone-Smith and Nickoloff, 1996; Gilhar *et al.*, 1997). We found that activated primary CD4 T cells are more sensitive to LtxA than resting cells using CD25 as a marker for T-cell activation. The difference in sensitivities between the three donors that we observed likely represents the natural

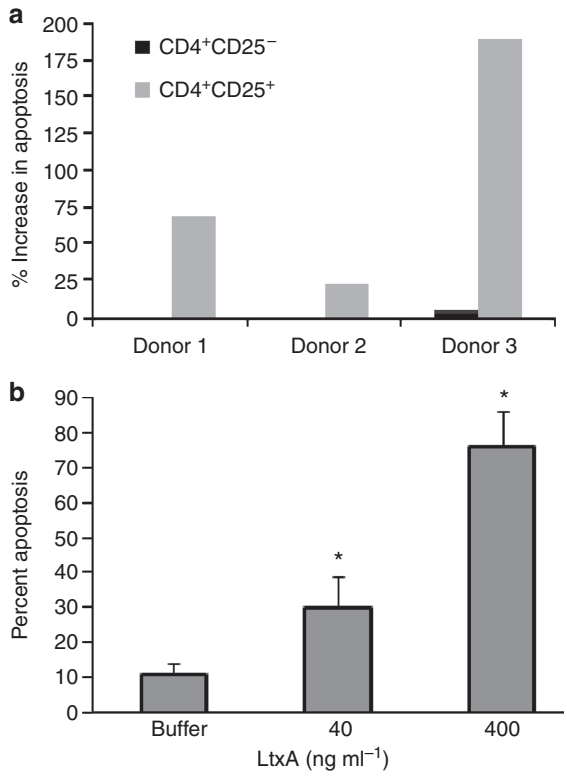

Figure 3. Susceptibility of white blood cells to leukotoxin (LtxA). (**a**) Effects of LtxA on activated T cells. Peripheral blood mononuclear cells (PBMCs) from three different human donors were treated with LtxA (40 ng ml−1) for 2 hours. Cells were stained with anti-CD4/CD25 antibodies and Annexin V (indicating apoptosis) and analyzed using flow cytometry. All samples were normalized to buffer treatment, and at least 20,000 cells were analyzed per sample. The values of $CD4^+CD25^-$ cells from donors 1 and 2 were slightly less than 1. (**b**) LtxA-induced apoptosis of proinflammatory monocytes. PBMCs were treated with LtxA for 24 hours and then stained with anti-CD14/CD16 antibodies and Annexin V, and analyzed by flow cytometry. Data represent mean ± SEM from three independent donor samples. *$P<0.05$.

variation of activation states of human clinical samples. Furthermore, it is possible that a T cell is $CD25^-$ but expresses an activated form of LFA-1 and the inverse. These results suggest that the activated WBCs responsible for disease in psoriasis patients would be preferential targets for LtxA. It is important to point out that WBCs lacking LFA-1 are not affected by LtxA (Lally *et al.*, 1997; Kachlany *et al.*, 2010). In addition to lymphocytes, macrophages are also implicated in disease. Presentation of antigen to T cells by macrophages can trigger a Th1-type cellular immune reaction that leads to psoriatic lesions (Baker *et al.*, 2001; Baker *et al.*, 2006), and local elimination of macrophages has led to resolution of cutaneous inflammation (Thepen *et al.*, 2000). $CD16^+$ monocytes constitute the proinflammatory subset of monocytes and are increased in patients with psoriasis and psoriatic arthritis (Chiu *et al.*, 2010), rheumatoid arthritis (Baeten *et al.*, 2000), and other inflammatory conditions (Ziegler-Heitbrock, 2007). These cells have been shown to produce higher amounts of tumor necrosis factor-α, can differentiate into dendritic cells, and possess proinflammatory properties that contribute to the pathogenesis of inflammatory diseases (Strauss-Ayali *et al.*, 2007). We show that $CD16^+$ monocytes were highly sensitive to LtxA, suggesting that these cells could also be targeted during treatment.

Efalizumab is an anti-LFA-1 monoclonal antibody therapy that was approved for use in psoriasis patients. As a comparator, we have used efalizumab because it addresses the same target as LtxA. The drug was recently removed from the market because of the increased risk of progressive multifocal leukoencephalopathy, a rare viral disease characterized by inflammation of the brain, during extended use. Although LtxA also targets LFA-1, there are important pharmacological differences between LtxA and efalizumab. First, LtxA is a naturally-occurring (non-engineered and non-recombinant) protein produced by a bacterium that is present in the oral cavity of a large percentage of the healthy population (Leys *et al.*, 1994; Lamell *et al.*, 2000). Clinically, efalizumab does not cause depletion of lymphocytes or other WBCs, but inhibits T-cell activation in the lymph nodes and binding of T cells to endothelial cells (Jullien *et al.*, 2004). In contrast, LtxA preferentially targets and depletes WBCs with activated LFA-1. Because of this preference for WBCs expressing activated LFA-1, most of the resting immune cells would be spared after LtxA treatment. For example, when administered intravenously to a *Rhesus macaque*, LtxA caused transient depletion of WBCs (several hours), and the levels never dropped below 50% (Kachlany *et al.*, 2010). In addition to T cells, LtxA targets other WBCs that are involved in inflammatory disease, including proinflammatory monocytes. Hence, we would expect for LtxA to be a highly potent therapeutic agent because it acts on the multiple cell types that are involved in the pathogenesis of psoriasis. Indeed, we found here that LtxA was just as or more effective as efalizumab *in vivo*, however, at a 9-fold lower dose. Recently, Koszik *et al.* (Koszik *et al.*, 2010) reported that efalizumab induces a state hyporesponsiveness in T cells, and this effect may explain why prolonged use of the drug can lead to immunosuppression and increased risk of progressive multifocal leukoencephalopathy. The different mechanisms of action exhibited by these two LFA-1-targeting agents may explain the difference in efficacy and doses required in the models presented here.

In a similar study, the effect of inhibiting another integrin, very late antigen-1 (VLA-1), was investigated in psoriasis (Conrad *et al.*, 2007). Like LFA-1, VLA-1 is a heterodimeric integrin and is composed of CD94a and CD29. VLA-1 binds to collagen IV of the basement membrane in skin, and only when T cells have entered the epidermis, they express VLA-1. Blocking of VLA-1 prevented the accumulation of epidermal T cells and the development of psoriasis in the AGR psoriasis xenograft transplantation model where transplanted non-lesional skin from psoriasis patients spontaneously develops into psoriasis (Conrad *et al.*, 2007). This study further supports our therapeutic strategy of affecting subgroups of activated T cells by targeting WBC integrins.

Treatment with efalizumab results in an increase in circulating WBCs presumably because the drug blocks migration

of cells out of the blood (Jullien *et al.*, 2004; Harper *et al.*, 2008). Interestingly, we observed that intravenous administration of LtxA to a non-human primate resulted in an initial drop in WBC counts, followed by an increase in circulating WBCs within 24 hours (Kachlany *et al.*, 2010). We attributed this increase in WBC count to migration of cells from the tissue and into circulation to replenish depleted cells; however, we also believe that binding of LtxA at low doses to LFA-1 may be blocking migration of WBCs out of the blood and into the tissues, much like the effects of efalizumab. In the mouse model presented here, LtxA most likely exerts its anti-psoriasis effects by depleting the WBCs that are resident in the grafted human tissue. In a psoriasis patient, although systemic LtxA not only would target and deplete circulating activated WBCs but also would prevent their migration into the skin and subsequent inflammation and plaque formation. Although LtxA may have the potential to stimulate an immune response, in preliminary studies using Rhesus monkeys and immunocompetent mice, we did not detect neutralizing antibody against the protein (unpublished data). Nonetheless, more extensive experiments are required to fully determine the protein's immunogenic potential in humans.

In addition to psoriasis, LFA-1 has been shown to have a significant role in the pathogenesis of other autoimmune and inflammatory diseases. Patients with lupus have increased expression of LFA-1 on T cells (Takasaki *et al.*, 1999), and mice deficient in LFA-1 showed increased survival and reduced pathogenesis in a mouse model for lupus (Kevil *et al.*, 2004). Furthermore, treatment of mice with anti-LFA-1 monoclonal antibody alleviated symptoms of disease in experimental murine lupus models (Connolly *et al.*, 1994; Kootstra *et al.*, 1997). Watts *et al.* (2005) showed that LFA-1 is critically important for induction of inflammatory arthritis in mice and that blocking LFA-1 with monoclonal antibody ameliorated disease. In patients with multiple sclerosis, LFA-1 is highly expressed on immune cells in the blood and central nervous system (Lou *et al.*, 1997; Elovaara *et al.*, 2000), and treatment with methylprednisolone reduces levels of LFA-1 on monocytes and lymphocytes in multiple sclerosis patients (Elovaara *et al.*, 1998). In ulcerative colitis and Crohn's disease, leukocytes in the intestinal tissue have increased expression of LFA-1, which has a role in the migration of WBCs from circulation toward the colonic epithelium (Vainer *et al.*, 2000; Bernstein *et al.*, 2002). LFA-1 was also shown to be involved in the pathogenesis of type I diabetes in both humans and experimental animal models (Hasegawa *et al.*, 1994; Linn *et al.*, 1994; Somoza *et al.*, 1994; Mysliwiec *et al.*, 1999). Thus, LtxA may have significant therapeutic potential for the treatment of psoriasis and numerous other autoimmune and inflammatory diseases.

MATERIALS AND METHODS

LtxA purification

LtxA was purified from culture supernatants of A. *actinomycetemcomitans* strain NJ4500, as previously described (Diaz *et al.*, 2006). Protein was lyophilized in sterile vials and stored at −80 °C until used.

Isolation and treatment of PBMCs from psoriasis patients

PBMCs were obtained from 10 donors with severe plaque-type psoriasis, 3 women and 7 men, age 29–63 years (48 ± 13 years). PBMCs were isolated from whole-blood samples by centrifugation over a lymphoprep density gradient (density = 1.077 ± 0.001 g cm^{-3}) and viable cells counted. PBMCs were cultured in cell medium: RPMI-1640 medium containing 10% heat-inactivated human serum pool, and 1% penicillin/streptomycin/gentamycin, at 5% CO_2, 37° C, and 90% humidity. Cells were seeded at a concentration of 1×10^6 cells ml^{-1}; 100 µl (1×10^5 cells) was added to each well of a 96-well-plate. Cells were activated with staphylococcal enterotoxin B at a final concentration of 1.0 µg ml^{-1}. Staphylococcal enterotoxin B and test reagents were incubated for 72 hours. Proliferation was assessed by the CellTiter-Glo Viability Assay Kit (Promega, Madison, WI). Proliferation was evaluated as percent cell viability upon activation as compared with non-activated cultures.

Psoriasis xenograft transplantation model

Keratome skin biopsies of lesional skin were obtained after informed consent from two patients with severe plaque-type psoriasis. The patients' psoriasis was untreated for at least 1 month before the time of skin removal. Keratome skin biopsies were cut into smaller pieces before transplantation onto the back of anesthetized (subcutaneous injection of Ketaminol (ketamine, 100 mg kg^{-1}; Intervet, Skovlunde, Denmark) and Narcoxyl (xylazine, 10 mg kg^{-1}; Intervet)) SCID mice (female, 6- to 8-week of age, M&B Taconic, Laven, Denmark). After a healing period of 10 days, the animals were divided into separate treatment groups. A total of 24 mice were used in the study. Animals were allocated to two consecutive study series, each representing skin grafts from one individual psoriasis patient (total of two patients, 12 mice for each series). Each series was subdivided into four groups. One group in each series served as untreated control (two mice), whereas the other groups were allocated to treatment with LtxA (four mice), efalizumab (four mice), or LtxA vehicle (two mice). LtxA (0.5 mg kg^{-1} (4.4 nmol kg^{-1})), efalizumab (6 mg kg^{-1} (40 nmol kg^{-1})), and vehicle (tris buffer/NaCl) were administered once daily by intraperitoneal injection for 3 weeks. The LtxA dose was chosen based on a previous study where LtxA in a concentration of 2 mg kg^{-1} was administered, with a therapeutic effect in a mouse leukemia xenograft model (Kachlany *et al.*, 2010). The efalizumab dose was chosen based on previous studies in the psoriasis xenograft transplantation model where 6 mg kg^{-1} efalizumab or 10 mg kg^{-1} anti-ICAM-1 were administered to mice, demonstrating a therapeutic effect (Zeigler *et al.*, 2001; Boehncke *et al.*, 2005). Throughout the study, untreated mice appeared identical to the vehicle-treated mice, and so these animals were grouped together as the negative control.

During treatment, human psoriatic skin grafts were clinically assessed twice weekly and given a semiquantitative clinical psoriasis score according to the clinical signs: scaliness, induration, and erythema. The parameters were scored using the three-point scale: 0 = complete lack of cutaneous involvement; 1 = slight involvement; 2 = moderate involvement; and 3 = severe involvement. On this scale from 0 to 3, a maximal score of 3 represents severe scale, induration, and erythema of the psoriatic xenografts. After 3 weeks and after the final clinical assessment, the animals were killed, and

4-mm size punch biopsies were taken centrally from each human psoriatic skin graft. We assessed epidermal thickness and presence of lymphocytes on five hematoxylin and eosin-stained sections from paraffin-embedded punch biopsies. Epidermal thickness was measured as the distance from stratum corneum to the deepest part of the rete pegs. Lymphocytes were evaluated and given a score in the range 0–4, where 0 denotes no psoriasis and 4 denotes full psoriasis. All sections were blinded before evaluation, and sections were evaluated randomly.

Statistical analyses

Results for Figure 1 are shown as mean ± SD. Results for Figures 2 and 3 are shown as mean ± SEM. The non-parametric Mann–Whitney test was used to test for differences between treatment groups in semiquantitative clinical psoriasis scores and lymphocyte scores. Student's *t*-test was used to test for no differences between treatment groups for WBC proliferation, epidermal thickness, and monocyte apoptosis. Observations made for different mice were assumed to be independent of each other. All tests were two-sided, and *P*-values < 0.05 were considered significant.

Isolation of PBMCs from healthy subjects

Human peripheral blood 'leukocyte units' from anonymous donors were provided by the New York Blood Center. PBMCs were isolated by ficoll density gradient centrifugation and resuspended in RPMI 1640 media containing 10% fetal bovine serum.

Treatment with leukotoxin and flow cytometry

Both activated and non-activated PBMCs (1×10^6 cells ml^{-1}) were cultured in the presence of LtxA (0.4–400 ng ml^{-1}) or buffer for 2 hours at 37 °C. Surface markers for monocytes and activated T cells were detected via flow cytometry by staining 1×10^6 cells on ice with anti-human antibodies CD14 allophycocyanin, CD16 PE-Cy5, CD4 PE, and CD25 PE-Cy5 (BioLegend, San Diego, CA). For detection of apoptosis, cells were subsequently stained with Annexin V fluorescein isothiocyanate or Annexin V Alexa Fluor 647 (BioLegend), incubated on ice in the dark for 15 minutes, and resuspended in 1× Annexin V binding buffer according to the manufacturer's protocol. At least 20,000 events in the live gate were acquired on the LSR II using FACSDiva Software 6.0 (BD Biosciences, Franklin Lakes, NJ). Data was analyzed using either FACSDiva Software or FlowJo Software (Tree Star, Ashland, OR).

Human and animal protocol approvals

Human psoriasis studies were approved by the Central Denmark Region Committees on Biomedical Research. All patients consented to graft donation in writing. The animal studies were carried out in the Animal Facility of the University of Aarhus, with approval from the Danish Experimental Animal Inspectorate. The protocol for use of PBMCs from healthy subjects was approved by the University of Medicine and Dentistry of New Jersey Institutional Review Board (Newark, NJ). All human studies were performed in accordance with the Declaration of Helsinki Principles.

CONFLICT OF INTEREST

BAB and SCK are founders of a company (Actinobac Biomed) that has licensed the technology for the therapeutic use of LtxA.

ACKNOWLEDGMENTS

We thank Amy Le for expert technical assistance during purification of LtxA and Vinni Bredahl for excellent technical assistance with the processing of biopsies.

REFERENCES

Abraham C, Griffith J, Miller J (1999) The dependence for leukocyte function-associated antigen-1/ICAM-1 interactions in T cell activation cannot be overcome by expression of high density TCR ligand. *J Immunol* 162:4399–405

Baeten D, Boots AM, Steenbakkers PG *et al.* (2000) Human cartilage gp-39+, CD16+ monocytes in peripheral blood and synovium: correlation with joint destruction in rheumatoid arthritis. *Arthritis Rheum* 43: 1233–43

Baker BS, Brown DW, Fischetti VA *et al.* (2001) Skin T cell proliferative response to M protein and other cell wall and membrane proteins of group A streptococci in chronic plaque psoriasis. *Clin Exp Immunol* 124:516–21

Baker BS, Laman JD, Powles A *et al.* (2006) Peptidoglycan and peptidoglycan-specific Th1 cells in psoriatic skin lesions. *J Pathol* 209:174–81

Bernstein CN, Sargent M, Rector E (2002) Alteration in expression of beta 2 integrins on lamina propria lymphocytes in ulcerative colitis and Crohn's disease. *Clin Immunol* 104:67–72

Boehncke WH, Ochsendorf FR, Noll S *et al.* (2005) Efficacy of the fully human monoclonal antibody MOR102 (#5) against intercellular adhesion molecule 1 in the psoriasis-severe combined immunodeficient mouse model. *Br J Dermatol* 153:758–66

Camacho SA, Heath WR, Carbone FR *et al.* (2001) A key role for ICAM-1 in generating effector cells mediating inflammatory responses. *Nat Immunol* 2:523–9

Chirathaworn C, Kohlmeier JE, Tibbetts SA *et al.* (2002) Stimulation through intercellular adhesion molecule-1 provides a second signal for T cell activation. *J Immunol* 168:5530–7

Chiu YG, Shao T, Feng C *et al.* (2010) CD16 (FcRgammaIII) as a potential marker of osteoclast precursors in psoriatic arthritis. *Arthritis Res Ther* 12:R14

Connolly MK, Kitchens EA, Chan B *et al.* (1994) Treatment of murine lupus with monoclonal antibodies to lymphocyte function-associated antigen-1: dose-dependent inhibition of autoantibody production and blockade of the immune response to therapy. *Clin Immunol Immunopathol* 72:198–203

Conrad C, Boyman O, Tonel G *et al.* (2007) Alpha1beta1 integrin is crucial for accumulation of epidermal T cells and the development of psoriasis. *Nat Med* 13:836–42

Dam TN, Kang S, Nickoloff BJ *et al.* (1999) 1alpha,25-dihydroxycholecalciferol and cyclosporine suppress induction and promote resolution of psoriasis in human skin grafts transplanted on to SCID mice. *J Invest Dermatol* 113:1082–9

de Boer OJ, Wakelkamp IM, Pals ST *et al.* (1994) Increased expression of adhesion receptors in both lesional and non-lesional psoriatic skin. *Arch Dermatol Res* 286:304–11

Diaz R, Ghofaily LA, Patel J *et al.* (2006) Characterization of leukotoxin from a clinical strain of *Actinobacillus actinomycetemcomitans*. *Microb Pathog* 40:48–55

Elovaara I, Lalla M, Spare E *et al.* (1998) Methylprednisolone reduces adhesion molecules in blood and cerebrospinal fluid in patients with MS. *Neurology* 51:1703–8

Elovaara I, Ukkonen M, Leppakynnas M *et al.* (2000) Adhesion molecules in multiple sclerosis: relation to subtypes of disease and methylprednisolone therapy. *Arch Neurol* 57:546–51

Fingerle G, Pforte A, Passlick B *et al.* (1993) The novel subset of CD14+/CD16+ blood monocytes is expanded in sepsis patients. *Blood* 82:3170–6

Gilhar A, David M, Ullmann Y *et al.* (1997) T-lymphocyte dependence of psoriatic pathology in human psoriatic skin grafted to SCID mice. *J Invest Dermatol* 109:283–8

Harper EG, Simpson EL, Takiguchi RH *et al.* (2008) Efalizumab therapy for atopic dermatitis causes marked increases in circulating effector memory CD4+ T cells that express cutaneous lymphocyte antigen. *J Invest Dermatol* 128:1173–81

Hasegawa Y, Yokono K, Taki T *et al.* (1994) Prevention of autoimmune insulin-dependent diabetes in non-obese diabetic mice by anti-LFA-1 and anti-ICAM-1mAb. *Int Immunol* 6:831–8

Hogg N, Harvey J, Cabanas C *et al.* (1993) Control of leukocyte integrin activation. *Am Rev Respir Dis* 148:S55–9

Hogg N, Smith A, McDowall A *et al.* (2004) How T cells use LFA-1 to attach and migrate. *Immunol Lett* 92:51–4

Jakobsen M, Stenderup K, Rosada C *et al.* (2009) Amelioration of psoriasis by anti-TNF-alpha RNAi in the xenograft transplantation model. *Mol Ther* 17:1743–53

Jullien D, Prinz JC, Langley RG *et al.* (2004) T-cell modulation for the treatment of chronic plaque psoriasis with efalizumab (Raptiva): mechanisms of action. *Dermatology* 208:297–306

Kachlany SC (2010) Aggregatibacter actinomycetemcomitans leukotoxin: from threat to therapy. *J Dent Res* 89:561–70

Kachlany SC, Schwartz AB, Balashova NV *et al.* (2010) Anti-leukemia activity of a bacterial toxin with natural specificity for LFA-1 on white blood cells. *Leuk Res* 34:777–85

Kandula S, Abraham C (2004) LFA-1 on CD4+ T cells is required for optimal antigen-dependent activation *in vivo*. *J Immunol* 173:4443–51

Kevil CG, Hicks MJ, He X *et al.* (2004) Loss of LFA-1, but not Mac-1, protects MRL/MpJ-Fas(lpr) mice from autoimmune disease. *Am J Pathol* 165: 609–16

Kinashi T (2005) Intracellular signalling controlling integrin activation in lymphocytes. *Nat Rev Immunol* 5:546–59

Kootstra CJ, Van Der Giezen DM, Van Krieken JH *et al.* (1997) Effective treatment of experimental lupus nephritis by combined administration of anti-CD11a and anti-CD54 antibodies. *Clin Exp Immunol* 108:324–32

Korostoff J, Wang JF, Kieba I *et al.* (1998) Actinobacillus actinomycetemcomitans leukotoxin induces apoptosis in HL-60 cells. *Infect Immun* 66:4474–83

Korostoff J, Yamaguchi N, Miller M *et al.* (2000) Perturbation of mitochondrial structure and function plays a central role in Actinobacillus actinomycetemcomitans leukotoxin-induced apoptosis. *Microb Pathog* 29:267–78

Koszik F, Stary G, Selenko-Gebauer N *et al.* (2010) Efalizumab modulates T cell function both *in vivo* and *in vitro*. *J Dermatol Sci* 60:159–66

Kunz M (2009) Current treatment of psoriasis with biologics. *Curr Drug Discov Technol* 6:231–40

Lally ET, Kieba IR, Sato A *et al.* (1997) RTX toxins recognize a beta2 integrin on the surface of human target cells. *J Biol Chem* 272:30463–9

Lamell CW, Griffen AL, McClellan DL *et al.* (2000) Acquisition and colonization stability of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in children. *J Clin Microbiol* 38:1196–9

Leys EJ, Griffen AL, Strong SJ *et al.* (1994) Detection and strain identification of *Actinobacillus actinomycetemcomitans* by nested PCR. *J Clin Microbiol* 32:1288–94

Linn T, Strate C, Federlin K *et al.* (1994) Intercellular adhesion molecule-1 (ICAM-1) expression in the islets of the non-obese diabetic and low-dose streptozocin-treated mouse. *Histochemistry* 102:317–21

Lou J, Chofflon M, Juillard C *et al.* (1997) Brain microvascular endothelial cells and leukocytes derived from patients with multiple sclerosis exhibit increased adhesion capacity. *Neuroreport* 8:629–33

McGregor JM, Barker JN, Ross EL *et al.* (1992) Epidermal dendritic cells in psoriasis possess a phenotype associated with antigen presentation: *in situ* expression of beta 2-integrins. *J Am Acad Dermatol* 27:383–8

Mysliwiec J, Kretowski A, Kinalski M *et al.* (1999) CD11a expression and soluble ICAM-1 levels in peripheral blood in high-risk and overt type 1 diabetes subjects. *Immunol Lett* 70:69–72

Nestle FO, Kaplan DH, Barker J (2009) Psoriasis. *N Engl J Med* 361: 496–509

Nickoloff BJ (2000) Characterization of lymphocyte-dependent angiogenesis using a SCID mouse: human skin model of psoriasis. *J Investig Dermatol Symp Proc* 5:67–73

Poulin Y, Langley RG, Teixeira HD *et al.* (2009) Biologics in the treatment of psoriasis: clinical and economic overview. *J Cutan Med Surg* 13(Suppl 2):S49–57

Rosada C, Stenderup K, de Darko E *et al.* (2010) Valrubicin in a topical formulation treats psoriasis in a xenograft transplantation model. *J Invest Dermatol* 130:455–63

Somoza N, Vargas F, Roura-Mir C *et al.* (1994) Pancreas in recent onset insulin-dependent diabetes mellitus. Changes in HLA, adhesion molecules and autoantigens, restricted T cell receptor V beta usage, and cytokine profile. *J Immunol* 153:1360–77

Stenderup K, Rosada C, Worsaae A *et al.* (2009) Interleukin-20 plays a critical role in maintenance and development of psoriasis in the human xenograft transplantation model. *Br J Dermatol* 160:284–96

Sterry W, Bagot M, Ferrandiz C *et al.* (2009) Immunosuppressive therapy in dermatology and PML. *J Dtsch Dermatol Ges* 7:5

Strauss-Ayali D, Conrad SM, Mosser DM (2007) Monocyte subpopulations and their differentiation patterns during infection. *J Leukoc Biol* 82:244–52

Takasaki Y, Abe K, Tokano Y *et al.* (1999) The expression of LFA-1, ICAM-1, CD80 and CD86molecules in lupus patients: implication for immunotherapy. *Intern Med* 38:175–7

Thepen T, van Vuuren AJ, Kiekens RC *et al.* (2000) Resolution of cutaneous inflammation after local elimination of macrophages. *Nat Biotechnol* 18:48–51

Todd I, Radford PM, Ziegler-Heitbrock L *et al.* (2007) Elevated CD16 expression by monocytes from patients with tumor necrosis factor receptor-associated periodic syndrome. *Arthritis Rheum* 56:4182–8

Vainer B, Nielsen OH, Horn T (2000) Comparative studies of the colonic *in situ* expression of intercellular adhesion molecules (ICAM-1, -2, and -3), beta2 integrins (LFA-1, Mac-1, and p150,95), and PECAM-1 in ulcerative colitis and Crohn's disease. *Am J Surg Pathol* 24: 1115–24

Valdimarsson H, Thorleifsdottir RH, Sigurdardottir SL *et al.* (2009) Psoriasis–as an autoimmune disease caused by molecular mimicry. *Trends Immunol* 30:494–501

van den Oord JJ, de Wolf-Peeters C (1994) Epithelium-lining macrophages in psoriasis. *Br J Dermatol* 130:589–94

Vestergaard C, Just H, Baumgartner Nielsen J *et al.* (2004) Expression of CCR2 on monocytes and macrophages in chronically inflamed skin in atopic dermatitis and psoriasis. *Acta Derm Venereol* 84:353–8

Villadsen LS, Schuurman J, Beurskens F *et al.* (2003) Resolution of psoriasis upon blockade of IL-15 biological activity in a xenograft mouse model. *J Clin Invest* 112:1571–80

Villadsen LS, Skov L, Dam TN *et al.* (2007) *In situ* depletion of CD4+ T cells in human skin by Zanolimumab. *Arch Dermatol Res* 298: 449–55

Watts GM, Beurskens FJ, Martin-Padura I *et al.* (2005) Manifestations of inflammatory arthritis are critically dependent on LFA-1. *J Immunol* 174:3668–75

Wrone-Smith T, Nickoloff BJ (1996) Dermal injection of immunocytes induces psoriasis. *J Clin Invest* 98:1878–87

Zeigler M, Chi Y, Tumas DB *et al.* (2001) Anti-CD11a ameliorates disease in the human psoriatic skin-SCID mouse transplant model: comparison of antibody to CD11a with cyclosporin A and clobetasol propionate. *Lab Invest* 81:1253–61

Ziegler-Heitbrock L (2007) The CD14+ CD16+ blood monocytes: their role in infection and inflammation. *J Leukoc Biol* 81:584–92